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(54) Title: NOVEL THERAPY FOR TREATMENT OF LYSOSOMAL STORAGE DISEASE

(57) Abstract

A method for treating or preventing a neurological disease, where the disease is characterized by deficiency of a factor, comprising administering to the CNS of an animal in need thereof, an effective amount of microcapsules which deliver the deficient factor.

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Title: NOVEL THERAPY FOR TREATMENT OF LYSOSOMAL STORAGE DISEASE

This application claims benefit from United States provisional application serial no. 60/084,413 filed on May 6, 1998.

FIELD OF THE INVENTION

The invention relates to a method for treatment of neurological diseases characterized by deficiency of a factor and in particular concerns a method for treating neurological sequelae of lysosomal storage disease.

BACKGROUND OF THE INVENTION

Over 30 different lysosomal storage diseases, each associated with deficiency of a specific lysosomal enzyme, have been described in man (Gieselmann, 1995). Because of the ubiquitous presence of lysosomes in almost all cell types, deficiencies of these enzymes cause multi-system anomalies, often with catastrophic consequences, and frequently result in early death. For example, mucopolysaccharidosis type VII (MPS VII) is an autosomal recessive disorder caused by deficient β-glucuronidase activity, resulting in the failure to cleave the terminal β-glucuronic acid from various glycosaminoglycans, e.g. chondroitin sulfate, dermatan sulfate, heparin sulfate, which accumulate in the lysosomes. Mutant mice which are models for this disease show characteristic dysmorphic facial features, abnormal gait, skeletal and joint abnormalities, dwarfism, organomegaly and a shortened life span (Birkenmeier et al., 1989; Vogler et al., 1990). The mutation is caused by a single base deletion in exon 10, resulting in a premature stop codon and complete absence of translation product, thus accounting for the total enzyme deficiency (Sands & Birkenmeier, 1993).

Although the incidence of individual enzyme deficiency is rare, collectively, lysosomal storage diseases account for 1 in ~10,000 births. In spite of such significant prevalence and devastating consequences, there is no cure or even definitive treatment for most of these diseases. Until recently, bone marrow transplantation has been the only experimental treatment with some degree of success (Krivit et al., 1990; Hoogerbrugge et al., 1995). However, this procedure carries high rate of morbidity (graft-*vs*.-host disease) and mortality (10% in matched and 20-25% in unmatched donors). Furthermore, even if the risks are acceptable, most patients (70%) cannot find compatible donors (Parkman, 1986). In the majority of the affected families, the only medical interventions available are palliative care until death occurs and prevention of recurrence through prenatal diagnosis. An alternative treatment available to a subtype of one form of lysosomal storage disease is enzyme replacement. For the non-neuropathic form of Gaucher disease, administration of glucocerebrosidase, the deficient enzyme in question, has resulted in definite clinical improvements. This therapy is now an accepted form of treatment in the U.S., but only for those who can afford it. Under the Orphan Drug protection law with no competitive markets, the annual cost for the enzyme Ceredase alone

is $380,000/70 kg (FDA recommendation, 1991). Because of the risks and the difficulty in locating donors for bone marrow transplant, and the high cost for both bone marrow transplantation (>$100,000) or enzyme replacement treatment, more cost-effective therapies for the lysosomal storage diseases need to be developed. Since the genes for many lysosomal enzymes have been cloned, somatic gene therapy for lysosomal storage diseases may offer the ultimate solution to this serious health care problem (Beutler, PNAS 90:5384-5390 (1993)).

Experimental Approaches to Therapy of Lysosomal Storage Diseases.

There are three experimental treatment protocols potentially available for these disorders: bone marrow transplantation, enzyme replacement and gene therapy.

Bone Marrow Transplantation

As inferred above, this approach has been tried in patients with various lysosomal storage diseases (Parkman, 1986; Krivit et al., 1992), *e.g.* metachromatic leukodystrophy (Krivit et al., 1990; Ladisch et al., 1986), Type I Gaucher (Rappeport & Ginns, 1984) and Hurler disease (Krivit & Whitley, 1987). Clinical improvements such as diminished visceromegaly (Hurler) and relief from bone pain (Gaucher) have been observed after allogenic bone marrow transplantation. The disadvantages of this treatment, as noted before, are the high risks and costs, and the difficulty in securing matched donors.

Enzyme Replacement.

Supplying purified enzyme to circumvent the enzyme deficiency is the basis of enzyme replacement therapies. In spite of early failures of this approach when exogenously enzymes were quickly cleared from the circulatory system with no observable clinical benefit (Bergsma et al., 1973; Desnick, 1979), recent refinements of this strategy have produced remarkable clinical improvements in patients with the Type 1 non-neuronopathic form of Gaucher disease (Grabowski 1993). Reduction in bone pain, organomegaly and increase in hematocrit are some of the clearly obtainable improvements. However, because of the high and, for many patients, unrealistic cost of ceredase treatment, alternate therapeutic strategies are desperately needed (Beutler, PNAS 90:5384-5390 (1993)), particularly for the remaining lysosomal storage diseases whose relevant enzymes have not been purified at the industrial scale.

Gene Therapy

Virus mediated

Gene therapy is theoretically available for many lysosomal storage diseases as many genes encoding lysosomal enzymes have been cloned. It offers a more direct approach to therapy as the high cost of protein purification required for enzyme replacement is eliminated. The correction of the enzyme deficiency in human Gaucher's affected cells has been reported in vitro after retroviral-mediated gene transfer (Fink et al, 1990) and human clinical trials are currently underway with retrovirally transduced $CD34^+$

enriched cells (Nimgaonkar et al. Cold Spring Habor Lab. Meeting on Gene Therapy, p. 7 (1994)) and Mucopolysaccharidosis type 1 with retrovirally transduced autologous fibroblast implants (Salvetti et al., *Hum. Gene. Ther.* 6:1153-1159 (1995)). A recombinant HSV-1 vector capable of infecting and being propagated in a non-mitotic cell as a vehicle to treat neurological diseases has also been described (Geller, A.I, *et al.* U.S. 5,501,979).

Microencapsulation

Cell lines that produce desired therapeutic products may be implanted into patients requiring the same product replacement (Chang, in Somatic Gene Therapy (ed. P.L. Chang) CRC, Florida, Chap. 12 (1995)) . To avoid immune rejection by the host, the non-autologous cells are enclosed in immuno-isolation devices based on the technology developed for allogenic or xenogeneic transplants. As such, the need for patient-specific genetic engineering is removed and the cost of treatment should be reduced.

By using a "universal" donor cell line engineered to produce a desired therapeutic product, the feasibility of this idea has been demonstrated *in vitro* and *in vivo*. *In vitro* studies showed that genetically-modified fibroblasts remained viable within these immuno-protective microcapsules (Chang et al., 1994) and continued to deliver recombinant gene products such as human growth hormone, factor IX (Liu et al., 1993) and lysosomal enzymes (Awrey D. et al., manuscript submitted (1995)).

In vivo studies have shown successful delivery of recombinant products such as human growth hormone (Chang et al., 1993), and human factor IX (Gonzalo et al., 1996) into the systemic circulation of rodents for several months. The clinical efficacy of this strategy was also proven by the correction of the growth retardation of the Snell dwarf mice suffering from growth hormone deficiency (Al-Hendy et al., 1995). However this form of therapy has not been used or proven in the central nervous system (CNS). To date only recombinant virus vectors capable of infecting and being propagated in a non-mitotic cells have been tried as a vehicle to treat neurological diseases (Geller, A.I, *et al.* U.S. 5,501,979). For example, virus directed gene therapy for lysosomal storage diseases has been tried on gus^{mps}/gus^{mps} mice (which are a known model of the human lysosomal storage disease mucopolysaccharidosis type VII (MPS VII) characterized by deficiency of β-glucuronidase) via direct viral infection of the CNS (Wolfe et al., 1992b). The results demonstrate biochemical and histological improvements, resulting in diminished lysosomal storage and restoration of low level of β-glucuronidase activity, but there has been no report of improved neurological function.

Other attempts at introducing β-glucuronidase directly into the CNS are reviewed by Sly and Volgler (*Nat. Med.* 3:719-720 (1997)) and include the following. Herpes infected cells expressing β-glucuronidase were found in the trigeminal ganglia and brainstems for up to four months (Wolfe et al., 1992), and adenoviral mediated gene-transfer resulted in detectable β-glucuronidase activity near the implant site and scattered in the parenchyma for at least 2 weeks (Ohashi et al., *Proc. Natl. Acad. Sci.* USA

94:1287-1292 (1997). Further, immortalized neural progenitor cells expressing β-glucuronidase transplanted into the cerebral ventricles of newborn mice showed an overall average of 11 % of normal β-glucuronidase activities throughout the CNS in mutant mice with successful grafts (Snyder et al., 1995). Implanted retroviral-corrected fibroblasts delivered up to 4.7 % of normal levels of β-glucuronidase adjacent to the graft site, and showed a clearance of lysosomal distention in neurons and glia near the graft site (Taylor and Wolfe, 1997). These approaches however, are difficult to carry out and, where the procedure involves a viral vector, success is dependent on the level and accuracy of viral infection of neural cells. As well, the infection is not necessarily limited to neural cells .

SUMMARY OF THE INVENTION

The present inventors have found that neurological function in a lysosomal storage disease characterized by enzyme deficiency is improved through direct delivery of microcapsules to the CNS of an affected animal, to deliver an enzyme that is deficient in the lysosomal storage disease.

A recent study by O'Connor L. H., *et al.* (J. Clin. Invest. 101: 1394-1400 (1998)) has demonstrated an improvement in behaviour and auditory function in Gus^{mps}/Gus^{mps} mice, receiving intravenous injection of recombinant β-glucurondiase. However, weekly injections were required and the treatment was started at birth: both aspects make application of the approach difficult in practice. Furthermore, the blood-brain barrier appears to restrict entry of β-glucurondiase into the CNS, consequently, it is unclear whether any improvement of the neurological lesions associated with the disease can be alleviated by this approach. Surprisingly, through direct delivery of microcapsules to the CNS of an affected animal to deliver an enzyme that is deficient in the lysosomal storage disease, the inventors have found significant improvement in behaviour and lysosomal storage lesions where an effective amount of microcapsules which deliver β–glucuronidase are injected directly into the cerebral ventricles of an animal in need of treatment of the neurological disease associated with MPS VII. This result is surprising in light of previous gene therapy work which infers that replacement β-glucuronidase in the CNS should be derived from neural cells.

Consequently, in its broad aspect the present invention provides a method for treating, or preventing, a neurological disease, where the disease is characterized by deficiency of a factor, comprising administering to the CNS of an animal in need thereof, an effective amount of microcapsules which deliver the deficient factor.

According to an embodiment, the present invention provides a method of treating neurological diseases where an enzyme, or other factor is deficient.

According to a preferred embodiment, the present invention provides a method of treating an animal having a neurological disease associated with any one of the lysosomal storage diseases, including the neuropathic form of Gaucher disease; MPS-I - Hurler and Scheie syndromes; MPS-II - Hunter syndrome; MPS-III - Sanfilippo syndrome

type B; MPS VI - Maroteaux-Lamy syndrome; a-manosidosis; Neimann-Pick; Tay-Sach and Sandhoff disease; and preferably MPS VII MLD.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described in relation to the drawings in which:

Figure 1 is a photograph of microcapsules used for neural implantation shown 2 days post-encapsulation.

Figure 2 is a comparison of histograms illustrating various enzyme levels in the CNS of treated and control mutant mice.

Figure 3A shows the histology of the brain comparing mutant controls and CNS treated mutant brain.

Figure 3B shows toluidine blue stained sections from the brains of control mutant GUS mice and treated mutant GUS mice at weeks 3 and 7 post-implantation.

Figure 4 shows the circadian rhythm behavioural reversal of treated MPS VII mice.

DETAILED DESCRIPTION OF THE INVENTION

Therapeutic Methods of the Invention

As mentioned above, the method of the present invention may be used for the treatment of many neurological diseases.

The present invention provides a method for treating or preventing a neurological disease, where the disease is characterized by deficiency of a factor, comprising administering to the CNS of an animal in need thereof, an effective amount of microcapsules which deliver the deficient factor.

The term "effective amount" as used herein means an amount effective, at dosages and for periods of time necessary to achieve the desired result. The term "animal" as used herein includes all members of the animal kingdom including humans.

According to one embodiment, the present invention provides a method of treating neurological diseases where an enzyme or other factor is deficient, comprising administering to the CNS of an animal in need thereof an effective amount of microcapsules which deliver the deficient enzyme or other factor. Examples of neurological diseases which may be treated according to the present invention include the Lesch-Nyhan syndrome; Alzheimer amyloid; amyloid polyneuropathies; Alzheimer disease, Huntington disease, Parkinsonism, trichothiodystrophy, ichthyosis, and the lysosomal storage diseases.

According to another embodiment of the present invention there is provided a method for treating the neurological disease of lysosomal storage disease, comprising administering to the CNS of an animal in need thereof an effective amount of microcapsules which deliver an enzyme that is deficient in the lysosomal storage disease.

Examples of lysosomal storage diseases which may be treated according to the present invention include the neuropathic form of Gaucher disease; MPS-I - Hurler and Scheie syndromes; MPS-II - Hunter syndrome; MPS-III - Sanfilippo syndrome type B; MPS VI - Maroteaux-Lamy syndrome; α-manosidosis; Neimann-Pick; Tay-Sach and Sandhoff disease; mucopolysaccharidosis type IIIA, mucopolysaccharidosis type IIIC, mucopolysaccharidosis type IIID, sialic acid storage disease (SIASD); glycogen storage disease IIb, gangliosidosis, generalized GM1, type 1, fumarate hydratase, sulfatidosis, juvenile, and preferably MPS VII.

According to a further embodiment of the present invention there is provided a method for treating the lysosomal storage disease, MPS VII , comprising administering to the CNS of an animal in need thereof, an effective amount of microcapsules which deliver an enzyme that is deficient in MPS VII. The enzyme is preferably β–glucuronidase.

According to yet another embodiment of the present invention there is provided a method for treating the lysosomal storage disease, MPS VII, comprising administering to the CNS of an animal in need thereof, an effective amount of microcapsules which deliver β– glucuronidase. The administration of the microcapsules is preferably by injection directly into the CNS, more preferably by implantation in at least one neural ventricle. An alternate embodiment is intrathecal injection.

Microcapsules of the Invention

Microcapsules of the present invention delivering a desired factor may be prepared according to methods well known to those skilled in the art.

Preferably microcapsules of the present invention deliver a cell line which express a desired factor. The cell line may be autologous or non-autologous. In the case of autologous cells they may naturally express the factor, or in the case of both autologous and non-autologous they may be transformed to a transformant host cell according to standard recombinant procedures, for example, as described in Al-Hendy, A. *et al.* Human Gene Therapy 6: 165-175 (1995) and as briefly discussed below. Preferably the cell line is that of fibroblasts or myoblasts which have been transformed to express β-glucuronidase. Typical examples of recombinant vector construction and cell transfection may be found in Al-Hendy, A. *et al.* Human Gene Therapy 6: 165-175 (1995). The term "transformant host cell" is intended to include prokaryotic and eukaryotic cells which have been transformed or transfected with a recombinant expression vector. The terms "transformed with", "transfected with", "transformation" and "transfection" are intended to encompass introduction of nucleic acid (e.g. a plasmid or a vector) into a cell by one of

many possible techniques known in the art. Prokaryotic cells can be transformed with nucleic acid by, for example, electroporation or calcium-chloride mediated transformation. Nucleic acid can be introduced into mammalian cells via conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofectin, electroporation or microinjection. Suitable methods for transforming and transfecting host cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory press (1989)), and other such laboratory textbooks.

Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the factors of the invention may be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus), yeast cells or mammalian cells. Other suitable host cells can be found in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1991).

Microcapsules delivering host cells may be prepared according to standard procedures such as those described in Al-Hendy, A. et al. Human Gene Therapy 6:165-175 (1995) or in Enzymology 137:575-579 (1988). Preferably, the microcapsules are prepared with sodium alginate, preferably alginate-poly-L-lysine-alginate.

Pharmaceutical Compositions

The microspheres of the present invention may be formulated into pharmaceutical compositions for administration to subjects in a biologically compatible form suitable for administration *in vivo*. The compositions delivering the microspheres of the invention can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the microspheres is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in Remington's Pharmaceutical Sciences (Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., USA 1985). On this basis, the compositions include, albeit not exclusively, solutions of the microspheres in association with one or more pharmaceutically acceptable vehicles or diluents, and delivered in buffered solutions with a suitable pH and iso-osmotic with the physiological fluids.

Accordingly, in another aspect, the present invention provides a pharmaceutical composition comprising appropriate microspheres of the present invention in admixture with a suitable diluent or carrier. The compositions are useful in the treatment of neurological diseases in which the disease is characterized by deficiency of a factor.

In another aspect, the present invention provides a commercial package delivering as active ingredient the microspheres in a pharmaceutically acceptable vehicle, together with instructions for its use for the treatment of a neurological disease in which the disease is characterized by deficiency of a factor.

The following non-limiting examples are illustrative of the present invention:

EXAMPLES

Example 1

Gus^{mps}/Gus^{mps} *Mouse.*

This mouse model of the human lysosomal storage disease mucopolysaccharidosis type VII (MPS VII) (Sly et al., 1973), was discovered by Birkenmeier and his co-workers in 1989. Since then, it has been thoroughly characterized at the molecular (Sands & Birkenmeier, 1993), pathological (Birkenemeier et al., 1989, Vogler et al., 1990) and behavioural levels (Chang et al., 1994b), rendering it a superior animal model to develop strategies for treating lysosomal storage diseases.

Transgenic studies also proved that this model is a genuine counterpart of the human disease (Kyle et al., 1990). Transgenic mutants in which the *gus^{mps}/gus^{mps}* mice were genetically engineered at the embryonic stage to carry the normal human gene produced a completely normal phenotype. Hence, success in therapy produced in this mutant will be directly relevant to the human disease. These mutants have neurological deficits which can be measured with behavioral tests (Chang et al., 1994b).

β-Glucuronidase Delivery to the Central Nervous System

Mouse 2A50 fibroblasts expressing mouse β-glucuronidase enclosed in alginate-poly-L-lysine-alginate (APA) microcapsules (Figure 1) were implanted.

Intraventricular implantation of encapsulated fibroblasts secreting β-glucuronidase into the central nervous system (CNS) was examined as a means to supply β–glucuronidase to the CNS of MPS VII affected mice. Mouse 2A50 fibroblasts secreting β-glucuronidase encapsulated within small APA microcapsules (100 μm diameter), were implanted into the left and right lateral ventricles of MPS VII mice. β-glucuronidase was detected in the CNS at both week 3 and week 7 post-implantation (Figure 2). Levels ranged from 43 - 6143 % of normal at week 3, and 0.2 - 298 % of normal at week 7. The highest β-glucuronidase levels were located at the implantation sites, with corresponding decreases at further distances away from the implantation sites.There was a concomitant decreases in secondarily elevated lysosomal enzyme levels that are abnormally increased in affected mice, in regions of the CNS that received β-glucuronidase. Lysosomal storage lesions were reduced in the CNS near the implantation sites (Figures 3A and 3B).

Example 2

Behavioral change as a result of β-glucuronidase delivery

To examine any behavioral change as a result of β-glucuronidase delivery to the CNS, circadian rhythms of mice were examined before and after treatment as described in Example 1.

Circadian rhythms were examined by measuring mouse activity on wheels inside cages. Mice were initially placed in a 12 hour cycles of light and dark for 7 days to acclimatize the mice to the cages and wheels. Mice were then placed in complete darkness for 7-10 days to determine the "pre-treatment" circadian rhythms. Mice were treated and then returned to complete darkness to measure "post-treatment" circadian rhythms. Experimental mutant mice, treated with microcapsules delivering β-glucuronidase secreting fibroblasts, showed positive changes in circadian rhythms after treatment (Figure 4). Fragmentation was decreased and stability of onset was more consistent. Control mutant mice treated with microcapsules that delivered mouse 3521 MPS VII affected fibroblasts that do not secrete β-glucuronidase showed no change in circadian rhythms, only a slow progressive decline in overall activity over time, as observed in untreated mutant mice. Thus, encapsulated cell therapy of the MPS VII mouse CNS shows the potentially high levels of enzyme delivery from a genetically modified universal cell line specifically intended to secrete high levels of a specific gene product.

Encapsulating non-autologous cells into microcapsules has been shown to be effective in treating not only the somatic but also the behavioural abnormalities of lysosomal storage diseases. Instead of engineering autologous patient-specific cells as is currently practised (Anderson, 1995), a single cell line can be established as a standard reagent. It may be stored in liquid nitrogen permanently and retrieved to propagate when needed. It can be used for different patients with the same genetic disorder, thus reducing the health care cost and increasing the affordability of somatic gene therapy.

While the present invention has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the invention is not limited to the disclosed examples. To the contrary, the invention is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

FULL CITATIONS FOR REFERENCES REFERRED TO IN THE SPECIFICATION

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DETAILED FIGURE LEGENDS

Figure 1. Microcapsules enclosing 2A-50 cells. 2A-50 fibroblasts were encapsulated within alginate poly-L-lysine-alginate capsules. All cells were encapsulated as described by Chang et al. (1994). Microcapsules for neural implantation shown 2 days post-encapsulation were smaller (200 μm average diameter, secreting 500 nmol β-glucuronidase activity/10^6 cells/h). Variations in concentric air flow rate delivered to the tip of the needle in the encapsulation process was manipulated to create smaller microcapsules (4 L/min for large microcapsules, 8 L/min for small microcapsules). Mice were anesthetized and 5 μl of microcapsules (~150 small microcapsules or 2.5 x 10^3 cells) were implanted within each of the lateral ventricles of the CNS from a 10 μl capacity glass capillary pipette (Drummond). Scale bars 500 μm.

Figure 2. β-glucuronidase, β-hexaminidase, and α-galactosidase enzyme levels in the brains of treated mutant GUS mice at week 3 (n=3), week 5 (n=1), week 7 (n=2), and week 8 (n=1) post-implantation. All brains were snap frozen and sectioned into 12 regions from anterior to posterior in 1mm increments for each hemisphere of the brain. All data are means± SD. Normal control (+/+) and untreated control mutant GUS (-/-) enzyme levels are indicated. β-glucuronidase levels are displayed on a semi-log scale to show the wide variation in enzyme observed levels.

Figure 3A. Histology of brain. Toluidine blue stained sections of age-matched mutant control brain shows severe disease pathology of distended lysosomes (a). In CNS-treated mutant brain, 7 weeks post-implantation of microcapsules within the lateral ventricles of the CNS, there was a disappearance of disease pathology in all cells near the implantation sites (b). Original magnification 200X (a-d), 400X (a,b).

Figure 3B. CNS Histology. Toluidine blue stained sections from the brains of control mutant GUS mice (a, d, g, j) and treated mutant GUS mice at weeks 3 (b, e, h) and 7 (c, f, i) post-implantation. Control mutant GUS mice show extensive lysosomal storage disease pathology with distended lysosomes (white material). Storage material is found throughout the brain in many cell types (a, j) with many distended lysosomes in neurons (d, several large lysosomes) and cortical neurons (d, many smaller distended lysosomes) and perivascular distended lysosomes are evident (g). Treated mutant GUS mice show reduced lysosomal stage, especially in sections near the implantation site. Magnification 1000x (A-G, I) 400x (J-H).

Figure 4. Circadian rhythm behavioral reversal of treated MPS VII mice. Circadian rhythms of mice were examined in (a) normal, (b) before and (c) after treatment. The graphic analysis of circadian rhythms shows black boxed periods of activity compared to white periods of inactivity. The data reflect a period of 48 hours (left to right) to visualize the onset and termination of daily activity. Each line down represents one day. Normal mice (a) have a low fragmentation of daily activity and inactivity, stable onset and termination of activity, and a stable period. Mutant mice (b) have highly fragmented activity, and unstable onset and cessation of daily activity. Treated mice circadian rhythm's (c) were compared to pre-treatment patterns (b) for the same mouse to reduce the influence of individual variation. After treatment the mutant mice showed an increasingly less fragmented pattern and a more stable onset and cessation of activity.

We Claim:

1. A method for treating or preventing a neurological disease, wherein the disease is characterized by deficiency of a factor, comprising administering to the CNS of an animal in need thereof, an effective amount of microcapsules which deliver the deficient factor.

2. The method of claim 1 wherein the neurological disease is Lesch-Nyhan syndrome, Alzheimer amyloid, or amyloid polyneuropathies.

3. The method of claim 1 wherein the neurological disease is a lysosomal storage disease.

4. The method of claim 3 wherein the deficient factor is an enzyme.

5. The method according to claim 3 or 4 wherein the lysosomal storage disease is Gaucher disease; MPS-I - Hurler syndrome; Scheie syndromes MPS-II - Hunter syndrome; MPS-III - Sanflilippo syndrome type B; MPS VI - Maroteaux-Lamy syndrome; α-manosidosis; Neimann-Pick; Tay-Sach disease; Sandhoff disease; MLD; or MPS VII.

6. The method of claim 5 where the lysosomal storage disease is MPS VII.

7. The method of claim 6 where the enzyme is β–glucuronidase.

8. The method according to any one of claims 1-7 where the microcapsules are injected directly into the CNS.

9. The method according to any one of claims 1-8 where the microcapsules are implanted in at least one of the neural ventricles.

10. A method for treating a neurological disease which is lysosomal storage disease characterized by deficiency of an enzyme, comprising administering to the CNS of an animal in need thereof, an effective amount of microcapsules which deliver the deficient enzyme.

11. The method of claim 10 wherein the lysosomal storage disease is Gaucher disease; MPS-I - Hurler syndrome; Scheie syndromes MPS-II - Hunter syndrome; MPS-III - Sanflilippo syndrome type B; MPS VI - Maroteaux-Lamy syndrome; α-manosidosis; Neimann-Pick; Tay-Sach disease; Sandhoff disease; or MPS VII.

12. The method of claim 10 wherein the neurological disease is MPS VII.

13. The method according to claim 10 or 12 where the enzyme is β–glucuronidase.

14. The method according to any one of claims 10-13 where the microcapsules are injected directly into the CNS.

15. The method according to any one of claims 10-14 where the microcapsules are implanted in at least one the neural ventricles.

16. A method of improving behaviour deficits associated with MPS VII in an animal in need thereof comprising implanting in the neural ventricles of the animal microcapsules which deliver β-glucuronidase.

17. The use of a microcapsule containing a factor for treating or preventing a neurological disease, wherein the disease is characterized by deficiency of the factor.

18. The use of claim 17 wherein the neurological disease is Lesch-Nyhan syndrome, Alzheimer amyloid, or amyloid polyneuropathies.

19. The use of claim 17 wherein the neurological disease is a lysosomal storage disease.

20. The use of claim 19 wherein the deficient factor is an enzyme.

21. The use according to claim 19 or 20 wherein the lysosomal storage disease is Gaucher disease; MPS-I - Hurler syndrome; Scheie syndromes MPS-II - Hunter syndrome; MPS-III - Sanflilippo syndrome type B; MPS VI - Maroteaux-Lamy syndrome; α-manosidosis; Neimann-Pick; Tay-Sach disease; Sandhoff disease; MLD; or MPS VII.

22. The use of claim 21 where the lysosomal storage disease is MPS VII.

23. The use of claim 22 where the enzyme is β–glucuronidase.

24. The use according to any one of claims 17-23 where the microcapsules are injected directly into the CNS.

25. The use according to any one of claims 17-24 where the microcapsules are implanted in at least one of the neural ventricles.

26. The use of a microcapsule which contains an enzyme for treating a neurological disease which is a lysosomal storage disease characterized by deficiency of the enzyme.

27. The use of claim 26 wherein the lysosomal storage disease is Gaucher disease; MPS-I - Hurler syndrome; Scheie syndromes MPS-II - Hunter syndrome; MPS-III - Sanflilippo syndrome type B; MPS VI - Maroteaux-Lamy syndrome; α-manosidosis; Neimann-Pick; Tay-Sach disease; Sandhoff disease; or MPS VII.

28. The use of claim 26 wherein the neurological disease is MPS VII.

29. The use according to claim 26 or 28 where the enzyme is β–glucuronidase.

30. The use according to any one of claims 26-29 where the microcapsules are injected directly into the CNS.

31. The use according to any one of claims 26-30 where the microcapsules are implanted in at least one the neural ventricles.

32. A use of improving behaviour deficits associated with MPS VII in an animal in need thereof comprising implanting in the neural ventricles of the animal microcapsules which deliver β-glucuronidase.

33. The use of a microcapsule containing a factor in the preparation of a medicament for treating or preventing a neurological disease, wherein the disease is characterized by deficiency of the factor.

34. The use of claim 33 wherein the neurological disease is Lesch-Nyhan syndrome, Alzheimer amyloid, or amyloid polyneuropathies.

35. The use of claim 33 wherein the neurological disease is a lysosomal storage disease.

36. The use of claim 35 wherein the deficient factor is an enzyme.

37. The use according to claim 35 or 36 wherein the lysosomal storage disease is Gaucher disease; MPS-I - Hurler syndrome; Scheie syndromes MPS-II - Hunter syndrome; MPS-III - Sanflilippo syndrome type B; MPS VI - Maroteaux-Lamy syndrome; α-manosidosis; Neimann-Pick; Tay-Sach disease; Sandhoff disease; MLD; or MPS VII.

38. The use of claim 37 where the lysosomal storage disease is MPS VII.

39. The use of claim 38 where the enzyme is β–glucuronidase.

40. The use according to any one of claims 33-39 where the microcapsules are injected directly into the CNS.

41. The use according to any one of claims 33-40 where the microcapsules are implanted in at least one of the neural ventricles.

42. The use of a microcapsule which contains an enzyme in the preparation of a medicament for treating a neurological disease which is a lysosomal storage disease characterized by deficiency of the enzyme.

43. The use of claim 42 wherein the lysosomal storage disease is Gaucher disease; MPS-I - Hurler syndrome; Scheie syndromes MPS-II - Hunter syndrome; MPS-III - Sanflilippo syndrome type B; MPS VI - Maroteaux-Lamy syndrome; α-manosidosis; Neimann-Pick; Tay-Sach disease; Sandhoff disease; or MPS VII.

44. The use of claim 42 wherein the neurological disease is MPS VII.

45. The use according to claim 42 or 44 where the enzyme is β–glucuronidase.

46. The use according to any one of claims 42-45 where the microcapsules are injected directly into the CNS.

47. The use according to any one of claims 42-46 where the microcapsules are implanted in at least one the neural ventricles.

48. A use of improving behaviour deficits associated with MPS VII in an animal in need thereof comprising implanting in the neural ventricles of the animal microcapsules which deliver β-glucuronidase.

FIGURE 1

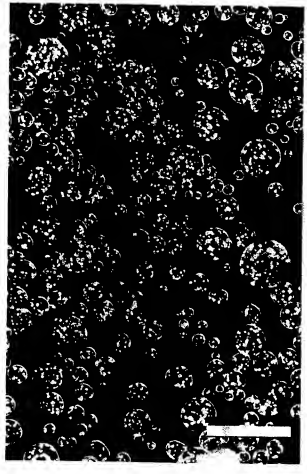
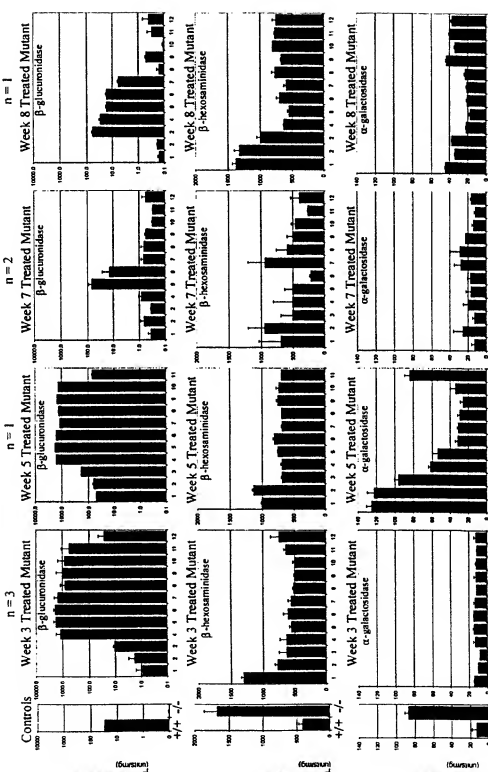


FIGURE 2


Controls
n = 3
n = 1
n = 2
n = 1
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Week 5 Treated Mutant
Week 7 Treated Mutant
Week 8 Treated Mutant
β-glucuronidase
β-glucosaminidase
β-hexosaminidase
α-galactosidase
β-Glucuronidase (units/mg)
β-Hexaminidase (units/mg)
α-Galactosidase (units/mg)
+/+ -/-
normal mutant
CNS Regions [#1] Anterior to [#12] Posterior, 1 mm increments

FIGURE 3A

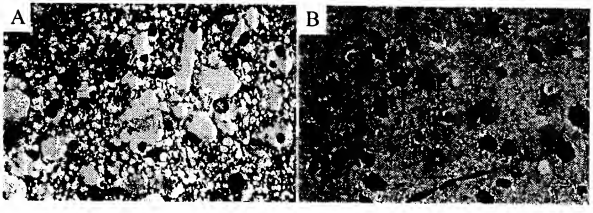


FIGURE 3B

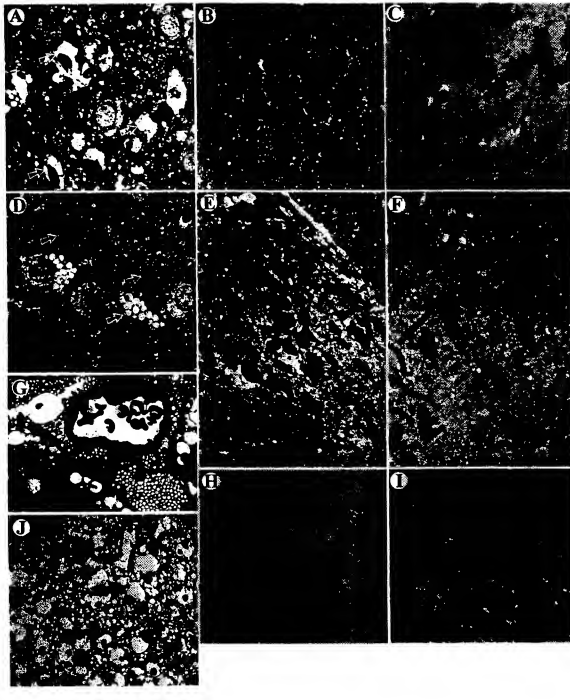
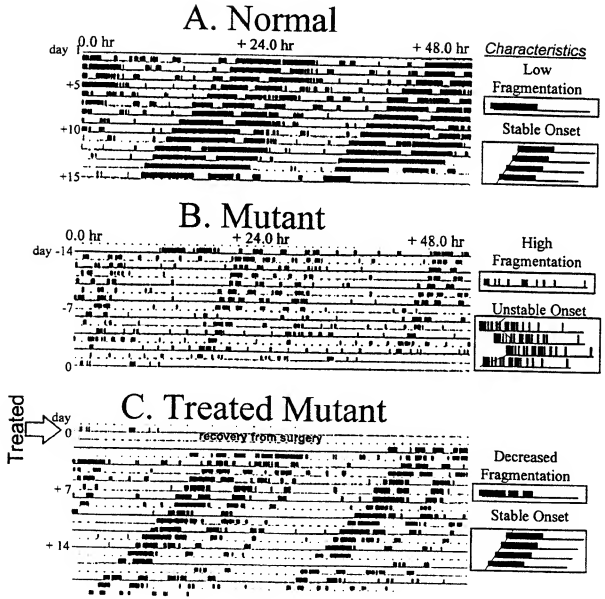


FIGURE 4


A. Normal
day 1
0.0 hr
+ 24.0 hr
+ 48.0 hr
+5
+10
+15
Characteristics
Low
Fragmentation
Stable Onset
B. Mutant
day -14
0.0 hr
+ 24.0 hr
+ 48.0 hr
-7
0
High
Fragmentation
Unstable Onset
Treated
C. Treated Mutant
day
0
recovery from surgery
+ 7
+ 14
Decreased
Fragmentation
Stable Onset

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/CA 99/00368

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/47 A61K9/50

According to international Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevent to claim No.
X	CHANG P L: "Microcapsules as bio-organs for somatic gene therapy." ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1997 DEC 31) 831 461-73, XP002122346 the whole document ---	1-48
Y	PEIRONE M A ET AL: "Delivery of recombinant gene product to canines with nonautologous microencapsulated cells." HUMAN GENE THERAPY, (1998 JAN 20) 9 (2) 195-206. , XP002122347 the whole document ---	1-48

-/--

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 November 1999	25/11/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/CA 99/00368

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	OHASHI T ET AL: "Adenovirus-mediated gene transfer and expression of human beta-glucuronidase gene in the liver, spleen, and central nervous system in mucopolysaccharidosis type VII mice." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 FEB 18) 94 (4) 1287-92. , XP002122348 the whole document	1-48
A	US 5 543 391 A (YATVIN M.B.) 6 August 1996 (1996-08-06) the whole document	1-40

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 99/00368

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-41 and 48 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

...formation on patent family members

Inter...nal Application No
PCT/CA 99/00368

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